

SHORT REPORT

Low frequency of *TERT* promoter mutations in gastrointestinal stromal tumors (GISTs)

Nathália C Campanella¹, Ricardo Celestino^{2,3}, Ana Pestana^{2,4}, Cristovam Scapulatempo-Neto^{1,5}, Antonio Talvane de Oliveira⁶, Maria José Brito⁷, António Gouveia⁸, José Manuel Lopes^{2,9,10}, Denise Peixoto Guimarães^{1,11}, Paula Soares^{2,10} and Rui M Reis^{*,1,12,13}

Somatic mutations in the promoter region of *telomerase reverse transcriptase* (*TERT*) gene, mainly at positions c. – 124 and c. – 146 bp, are frequent in several human cancers; yet its presence in gastrointestinal stromal tumor (GIST) has not been reported to date. Herein, we searched for the presence and clinicopathological association of *TERT* promoter mutations in genomic DNA from 130 bona fide GISTs. We found *TERT* promoter mutations in 3.8% (5/130) of GISTs. The c. – 124C>T mutation was the most common event, present in 2.3% (3/130), and the c. – 146C>T mutation in 1.5% (2/130) of GISTs. No significant association was observed between *TERT* promoter mutation and patient's clinicopathological features. The present study establishes the low frequency (4%) of *TERT* promoter mutations in GISTs. Further studies are required to confirm our findings and to elucidate the hypothetical biological and clinical impact of *TERT* promoter mutation in GIST pathogenesis. *European Journal of Human Genetics* advance online publication, 24 September 2014; doi:10.1038/ejhg.2014.195

INTRODUCTION

The *telomerase reverse transcriptase* (*TERT*) gene encodes the catalytic subunit of telomerase that is crucial to maintenance and regulation of the telomeres.^{1,2} In normal somatic adult tissues, telomerase activity is restricted to stem cells, and telomerase reactivation was proposed to be one of cancer hallmarks.³ Recently, hotspot somatic mutations in the promoter region of *TERT*, located – 124 and – 146 bp upstream from the ATG start site (c. – 124C>T and c. – 146C>T) were reported in several human cancers, including bladder (~85% of mutated cases), gliomas (~50%), thyroid (~15%) and melanoma (22–85%).^{4–9} It was proposed that both c. – 124C>T and c. – 146C>T mutations create new binding motif sites (GGAA) of ETS transcription factors leading to upregulation of *TERT* levels and protein activity.^{4,5}

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumor on the gastrointestinal tract characterized by hotspot mutations in *KIT* and *PDGFRA* genes, which are predictive of imatinib-based therapy response.^{10,11} Somatic *BRAF* mutations^{12–14} and germinative *SDHx* mutations were reported in a subset of *KIT*/*PDGFRA* wild-type GIST.^{15,16} Increased telomerase activity was reported in GISTs and was associated with poor prognosis.¹⁷ Yet, *TERT* promoter mutation has not been reported in GIST. Herein, we searched for the presence and clinicopathological association of the c. – 124C>T and c. – 146C>T *TERT* promoter mutations in a series of 130 bona fide GISTs.^{12,18–20}

MATERIALS AND METHODS

One hundred and thirty cases of GIST were selected from the files of the Department of Pathology from Barretos Cancer Hospital, Brazil, Centro

Hospitalar S. João and Garcia de Orta Hospital, Portugal. The cases were retrospectively re-evaluated and classified according to the WHO classification,²¹ and were assessed for the mean age, primary localization, tumor size, National Comprehensive Cancer Network (NCCN) risk classification,²² metastasis and overall survival. The mean age of the patients was 59.8 years, 52.3% were male and the tumors were located mainly in the stomach (50%) and the small intestine (32.7%). Most tumors had tumor size >5 cm, high malignancy risk and metastatic potential (Table 1).

The characterization of the mutational status for *KIT* and *PDGFRA* was performed in all GISTs.^{12,18–20} In addition, the *BRAF* mutation status was evaluated in *KIT*/*PDGFRA* wild-type GISTs (*n*=9) from Barretos Cancer Hospital and the *SDH* genes status was evaluated in *KIT*/*PDGFRA*/*BRAF* wild-type GISTs (*n*=18) from Centro Hospitalar S. João.^{15,16}

Tumor genomic DNA was extracted from formalin-fixed and paraffin-embedded tissues using the QIAamp DNA MicroKit (Qiagen, Hilden, Germany), following the manufacturer's instructions.¹⁹ A fragment of the *TERT* promoter was amplified with PCR using primers 5'-AGTGGATTCGCGGGCACAGA-3' and 5'-CAGCGCTGCCTGAAACTC-3', resulting in a PCR product of 235 bp, which contained the chr5.hg19:g.1295228C>T and Chr5.hg19:g.1295250C>T sites of mutations. Alternatively, gene mutations can be designated based on their upstream location to the ATG initiation codon of *TERT*, as c. – 124C>T, and c. – 146C>T, as previously described.⁷ PCR was performed with an initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C denaturation for 30 s, 64 °C annealing for 90 s and 72 °C elongation for 30 s and 72 °C final elongation for 7 min. Quality of PCR products was confirmed with gel electrophoresis. DNA sequencing of the PCR product was performed using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3500 xL Genetic Analyzer (Applied Biosystems). The chromatograms were compared with the reference sequence (GeneBank, *TERT*:

¹Molecular Oncology Research Center, Barretos Cancer Hospital, Sao Paulo, Brazil; ²Institute of Molecular Pathology and Immunology of University of Porto (IPATIMUP), Porto, Portugal; ³School of Allied Health Sciences ESTSP, Polytechnic of Porto, Porto, Portugal; ⁴Institute of Biomedical Sciences of University of Porto, Porto, Portugal; ⁵Department of Pathology, Barretos Cancer Hospital, Sao Paulo, Brazil; ⁶Department of Surgery, Barretos Cancer Hospital, Sao Paulo, Brazil; ⁷Department of Pathology, Hospital Garcia de Orta, Almada, Portugal; ⁸Department of Surgery, Hospital São João, Porto, Portugal; ⁹Department of Pathology, Centro Hospitalar de São João, Porto, Portugal; ¹⁰Department of Pathology and Oncology, Medical Faculty, University of Porto, Porto, Portugal; ¹¹Department of Endoscopy, Barretos Cancer Hospital, Sao Paulo, Brazil; ¹²Life and Health Sciences Research Institute (ICVS), Health Sciences School, University of Minho, Braga, Portugal; ¹³ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal *Correspondence: Dr RM Reis, Molecular Oncology Research Center, Barretos Cancer Hospital, Rua Antenor Duarte Vilela, 1331, CEP: 14784 400, Barretos, Sao Paulo CEP 14784 400, Brazil. Tel: +55 11 1733216600; Fax: +55 11 351 253 604850; E-mail: ruireis.hcb@gmail.com

Received 25 April 2014; revised 11 August 2014; accepted 20 August 2014

ENST00000310581). The SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used for all statistical analysis. To assess the relationship between variables, we used the Fisher's exact test. The *P*-value established for the statistics significance was <0.05 .

Table 1 Association of *TERT* promoter mutation status and clinicopathological and molecular features of GISTs

Variable	TERT wild type	TERT mutated	P-value
Mean Age	59.6	65.9	0.351
Primary localization			
Esophagus	1 (0.9)	0	0.132
Stomach	62 (53)	3 (60)	
Small intestine	37 (31.6)	0	
Large intestine	2 (1.7)	1 (20)	
Rectum	4 (3.4)	1 (20)	
Mesentery	2 (1.7)	0	
Retroperitoneum	6 (5.1)	0	
Esophagus/stomach	1(0.9)	0	
Other	2 (1.7)	0	
Tumor size			
≤ 5 cm	41 (36.3)	1 (20)	0.654
> 5 cm	72 (63.7)	4 (80)	
NCCN risk classification ^a			
Benign	2 (2.2)	0	0.646
Very low	19 (21.3)	0	
Low	18 (20.2)	2 (40)	
Intermediate	10 (11.2)	0	
High	40 (44.9)	3 (60)	
Metastasis			
Absent	78 (70.9)	4 (80)	1
Present	32 (29.1)	1 (20)	
Overall survival			
Dead	23 (27.4)	1 (20)	0.398
Alive	61 (72.6)	4 (80)	
KIT/PDGFRA/BRAF status			
KIT mutated	83 (66.4)	2 (40)	0.186
PDGFRA mutated	16 (12.8)	0	
Wild type	26 (20.8)	3 (60)	

Abbreviations: NCCN, National Comprehensive Cancer Network.

^aMiettinen and Lasota.²²

RESULTS

We found *TERT* promoter mutations in 3.8% (5/130) of the GISTs (Table 2 and Figure 1). The identified mutations are described in the LOVD database (<https://research.cchmc.org/LOVD2/home.php>; patient IDs 819–823). The c. –124C>T mutation was the most common event, present in 2.3% (3/130), and the c. –146C>T mutation in 1.5% (2/130) of GISTs. The two mutations occur in a mutually exclusive manner. No statistical correlation was found between *TERT* mutation and GIST clinical or molecular features (Table 1). Yet, *TERT* mutations appeared in tumors of slightly older patients, and no *TERT*-mutated cases were detected in benign/very-low malignancy risk GISTs (Table 1).

DISCUSSION

This study describes for the first time the occurrence of *TERT* promoter mutations (c. –124C>T and c. –146C>T) in GISTs, being present in ~4% of the cases. As paired blood or constitutive DNA of the tumors analyzed in the present study was not available, we cannot confirm the somatic nature of the c. –124 or c. –146 mutations identified. However, germline mutations at these hotspots were not reported in the various *TERT* studies that performed such paired (tumor *versus* normal) analysis.^{4–7,23–25} In addition, in the COSMIC database,²⁶ these mutations are described as somatic, and they are not present in the 1000 Genomes database.²⁷ Therefore, we can almost certainly assume that the mutations observed in GISTs were somatically acquired.

Previously, we analyzed a series of 36 GISTs and did not identify any *TERT* promoter mutation.⁷ Likewise, Killela *et al*⁶ also analyzed nine GISTs and did not found any *TERT* promoter mutation. As identical methodologies were used in all studies, one plausible reason for this discrepancy is the small number of cases analyzed in the previous studies.^{6,7}

TERT promoter mutations seem to be widespread in cancer, although showing tissue specificity. Killela *et al*⁶ suggested that cancers developing in tissues that are regularly self-renewing, such as in the gastrointestinal tract, skin and bone marrow, are not likely to harbor telomere-maintaining mutations, as telomerase is already epigenetically activated in their precursor cells. In contrast, cancers arising from cells that are not regularly self-renewing might harbor such mutations. GISTs fit to the second setting, as they are assumed to originate from the low-renewal Cajal cells or their precursors.²⁸ GISTs are prone to exhibit a high risk of disease relapse and metastasis spreading to distant organs such as the liver, peritoneal surface and lung.¹⁰ Previous reports associate telomerase activity in GIST with higher tumor malignancy risk, metastasis and worse prognosis.^{29–31} We found a low frequency of *TERT* mutations in GIST, but any

Table 2 Clinicopathological and molecular data of the GISTs with *TERT* promoter mutation (clinicopathological and molecular features of *TERT* promoter-mutated GISTs)

ID	Hospital	Age (years)	Gender	Primary localization	Tumor size (cm)	NCCN risk classification ^a	Metastasis	Status at last follow-up	Follow-up time (years)	<i>KIT</i> / <i>PDGFRA</i> / <i>BRAF</i> mutation status	<i>TERT</i> promoter mutation
Case 44	Barretos	77	Female	Stomach	7	Intermediate	Absent	Alive without cancer	10.71	<i>KIT</i>	c. –146C>T
Case 75	Barretos	47	Male	Rectum	4	Intermediate	Present	Alive with cancer	11.71	<i>KIT</i>	c. –124C>T
Case 159	S. João	49	Male	Stomach	6.5	High	Absent	Alive without cancer	22.63	Wild type	c. –124C>T
Case 215	S. João	76	Male	Stomach	6	Intermediate	Absent	Alive without cancer	8.78	Wild type	c. –124C>T
Case 216	S. João	81	Male	Large intestine	12.5	High	Absent	Death due to cancer	0.02	Wild type	c. –146C>T

Abbreviation: NCCN, National Comprehensive Cancer Network.

^aMiettinen and Lasota.²²

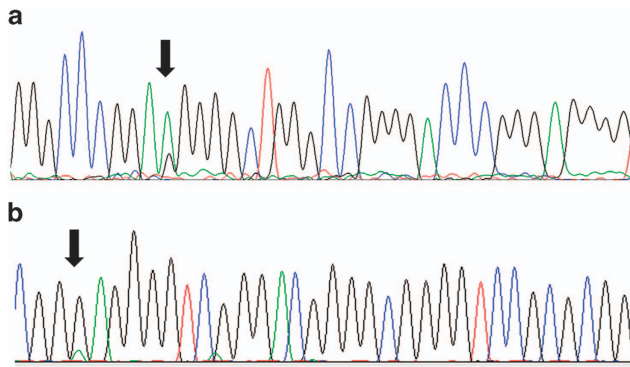


Figure 1 Electropherogram of *TERT* promoter mutations. (a) Heterozygotic c.-124C>T mutation (arrow); (b) Heterozygotic c.-146C>T mutation (arrow).

statistical association was found with tumor aggressiveness; however, most *TERT*-mutated GISTs displayed high recurrence risk features. Although our series is undersized to allow definitive conclusions, it would be of interest to further evaluate whether *TERT* promoter mutations associate with a higher expression of telomerase in GISTs, and to assess whether *TERT* promoter mutations associate with poor prognosis as reported in other cancers such as cancers of the thyroid,³² melanoma⁹ and brain.⁶

On the whole, our study establishes the presence of *TERT* promoter mutations in a subset of GISTs (4%). Future studies are required to validate our findings and to elucidate the potential biological and clinical impact of *TERT* promoter mutation in GIST pathogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This project was partially supported by Barretos Cancer Hospital internal research funds (PAIP) and CNPq Universal Grant (476192/2013-7) to RMR. NCC is a recipient of an FAPESP Doctoral Fellowship (2013/25787-3). Further funding from the project 'Microenvironment, metabolism and cancer' that was partially supported by Programa Operacional Regional do Norte (ON.2—O Novo Norte) under the Quadro de Referência Estratégico Nacional (QREN) and the Fundo Europeu de Desenvolvimento Regional (FEDER). IPATIMUP is an Associate Laboratory of the Portuguese Ministry of Science, Technology and Higher Education that is partially supported by the FCT.

- Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA: Highly recurrent *TERT* promoter mutations in human melanoma. *Science* 2013; **339**: 957–959.
- Killela PJ, Reitman ZJ, Jiao Y *et al*: *TERT* promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci USA* 2013; **110**: 6021–6026.
- Vinagre J, Almeida A, Populo H *et al*: Frequency of *TERT* promoter mutations in human cancers. *Nat Commun* 2013; **4**: 2185.
- Rachakonda PS, Hosen I, de Verdier PJ *et al*: *TERT* promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. *Proc Natl Acad Sci USA* 2013; **110**: 17426–17431.
- Pópulo H, Boaventura P, Vinagre J *et al*: *TERT* promoter mutations in skin cancer: the effects of sun exposure and irradiation. *J Invest Dermatol* 2014; **134**: 2251–2257.
- Campanella NC, de Oliveira AT, Scapulatempo-Neto C, Guimaraes DP, Reis RM: Biomarkers and novel therapeutic targets in gastrointestinal stromal tumors (GISTs). *Recent Pat Anticancer Drug Discov* 2013; **8**: 288–297.
- Joensuu H, Hohenberger P, Corless CL: Gastrointestinal stromal tumour. *Lancet* 2013; **382**: 973–983.
- Martinho O, Gouveia A, Viana-Pereira M *et al*: Low frequency of MAP kinase pathway alterations in KIT and PDGFRA wild-type GISTs. *Histopathology* 2009; **55**: 53–62.
- Agaimy A, Terracciano LM, Dirnhöfer S *et al*: V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild-type gastrointestinal stromal tumours. *J Clin Pathol* 2009; **62**: 613–616.
- Agaram NP, Wong GC, Guo T *et al*: Novel V600E BRAF mutations in imatinib-naïve and imatinib-resistant gastrointestinal stromal tumors. *Genes Chromosomes Cancer* 2008; **47**: 853–859.
- Celestino R, Lima J, Faustino A *et al*: A novel germline SDHB mutation in a gastrointestinal stromal tumor patient without bona fide features of the Carney-Stratakis dyad. *Fam Cancer* 2012; **11**: 189–194.
- Celestino R, Lima J, Faustino A *et al*: Molecular alterations and expression of succinate dehydrogenase complex in wild-type KIT/PDGFRA/BRAF gastrointestinal stromal tumors. *Eur J Hum Genet* 2013; **21**: 503–510.
- Sabah M, Cummins R, Leader M, Kay E: Expression of human telomerase reverse transcriptase in gastrointestinal stromal tumors occurs preferentially in malignant neoplasms. *Hum Pathol* 2004; **35**: 1231–1235.
- de Oliveira AT, Reis RM, Afonso J *et al*: Lymphangiogenic VEGF-C and VEGFR-3 expression in genetically characterised gastrointestinal stromal tumours. *Histol Histopathol* 2011; **26**: 1499–1507.
- de Oliveira AT, Pinheiro C, Longatto-Filho A *et al*: Co-expression of monocarboxylate transporter 1 (MCT1) and its chaperone (CD147) is associated with low survival in patients with gastrointestinal stromal tumors (GISTs). *J Bioenerg Biomembr* 2012; **44**: 171–178.
- Gomes AL, Gouveia A, Capelinha AF *et al*: Molecular alterations of KIT and PDGFRA in GISTs: evaluation of a Portuguese series. *J Clin Pathol* 2008; **61**: 203–208.
- Bosman FT CF, Hruban RH, Theise ND: *WHO Classification of Tumours of the Digestive System*. Lyon: IARC, Vol 3, 2010.
- Miettinen M, Lasota J: Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70–83.
- Griewank KG, Murali R, Schilling B *et al*: *TERT* promoter mutations in ocular melanoma distinguish between conjunctival and uveal tumours. *Br J Cancer* 2013; **109**: 497–501.
- Landa I, Ganly I, Chan TA *et al*: Frequent somatic *TERT* promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. *J Clin Endocrinol Metab* 2013; **98**: E1562–E1566.
- Nault JC, Mallet M, Pilati C *et al*: High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* 2013; **4**: 2218.
- Cosmic Database Home. (<http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>).
- Abecasis GR, Auton A, Brooks LD *et al*: An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491**: 56–65.
- Corless CL, Barnett CM, Heinrich MC: Gastrointestinal stromal tumours: origin and molecular oncology. *Nat Rev Cancer* 2011; **11**: 865–878.
- Sakurai S, Fukayama M, Kaizaki Y *et al*: Telomerase activity in gastrointestinal stromal tumors. *Cancer* 1998; **83**: 2060–2066.
- Kawai J, Kodera Y, Fujiwara M *et al*: Telomerase activity as prognostic factor in gastrointestinal stromal tumors of the stomach. *Hepatogastroenterology* 2005; **52**: 959–964.
- Wang Q, Kou YW: Study of the expressions of p53 and bcl-2 genes, the telomerase activity and apoptosis in GIST patients. *World J Gastroenterol* 2007; **13**: 2626–2628.
- Melo M, Rocha AG, Vinagre J *et al*: *TERT* promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. *J Clin Endocrinol Metab* 2014; **99**: E754–E765.

- Daniel M, Peek GW, Tollefsbol TO: Regulation of the human catalytic subunit of telomerase (hTERT). *Gene* 2012; **498**: 135–146.
- Heidenreich B, Rachakonda PS, Hemminki K, Kumar R: *TERT* promoter mutations in cancer development. *Curr Opin Genet Dev* 2013; **24C**: 30–37.
- Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.
- Horn S, Figl A, Rachakonda PS *et al*: *TERT* promoter mutations in familial and sporadic melanoma. *Science* 2013; **339**: 959–961.